



## Review Paper

# Exploitation alternatives of olive mill wastewater: production of value-added compounds useful for industry and agriculture

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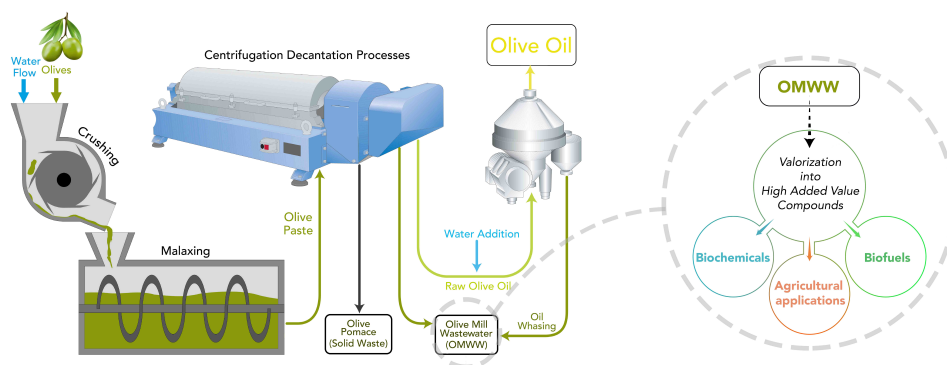
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## HIGHLIGHTS

- Olive mill wastewater (OMWW) can be exploited as feedstock for production of high value-added commodities.
- An overview on potential uses of OMWW and related valorization strategies is presented.
- A multifaceted approach integrating sustainable management and biorefinery concept is needed.
- Valorization of various waste streams including OMWW is the main challenge faced by olive oil industry.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Countries producing olive oil generate a considerable amount of olive mill wastewater (OMWW), one of the most harmful agro-industrial effluents with a powerful polluting capacity. In fact, owing to its high pollution load, this effluent is extremely toxic to the whole soil-air-water ecosystem as well as to the living organisms inhabiting it (i.e., plants, animals, aquatic organisms, microorganisms, etc.). Currently, OMWW is discarded but since it includes carbohydrates, organic acids and mineral nutrients, as well as elevated contents of phenolics and other natural antioxidants compounds, it could be considered as a potential source of high value-added natural products. Therefore, the valorization of different waste streams including OMWW into fine biochemicals and the recovery of valuable metabolites via biotechnological processes is probably the main challenge faced by the olive oil industry. In light of that, the aim of the present review article is to summarize the state-of-the-art in relation to the exploitation possibilities and the use of OMWW to generate added-value compounds of great significance for the biofuel, pharmaceutical, cosmetic, chemical, food, and agriculture industries. Valorization of this significant waste steam in particular through a biorefinery platform could substantially enhance the environmental sustainability aspects of the whole industry while simultaneously contributing to the improvement of its economic viability.

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## 1. Introduction

Valorization and recycling constitute new concepts which are increasingly necessary worldwide. Typically, by-products, wastes, and effluents from fruit and vegetable processing consist of high amounts of proteins, sugars, and lipids along with peculiar aromatic and aliphatic compounds; thus, they could be considered as cheap and abundant raw materials for the synthesis of value-added chemicals and biomaterials (Federici et al., 2009; Murthy and Naidu, 2012).

In relation to olive oil production, the chief concern is to find eco-friendly and economically viable solutions for the disposition and management of olive mill wastewater (OMWW). Considerable quantities of OMWW are produced during the manufacture of olives by the traditional milling and pressing processes, generating 1–2 t of OMWW during the processing of 1 t of olives (Paraskeva and Diamadopoulos, 2006). OMWW is a stable emulsion composed of vegetation water of olives, washing and process water, soft tissues from the olive pulp, and traces of olive. In many cases, direct disposal of olive mill effluents into lakes, rivers, and water streams has resulted in disastrous environmental consequences due to their high pollutant capacity (Yay et al., 2012).

Treatment of this agro-industrial effluent involves large capital investments and the installation of operating units that are of limited efficiency due to the high organic loads (chemical oxygen demand–COD– and biological oxygen demand–BOD). Moreover, the presence of organic and inorganic polluting substances makes this waste stream toxic to bacteria and other microorganisms used in biological treatments (El-Abbassi et al., 2012). However, the overall outlook is not completely discouraging and there are evidences highlighting the use of OMWW as an economic resource. In this sense, several studies have indicated that OMWW could be recycled and used as starting material to obtain profitable compounds such as antioxidants, enzymes, biogas, soil conditioners, feed and food, and fertilizers (Kourmentza et al., 2017; Gullón et al., 2018).

The present article critically reviews and discusses alternative approaches to

valorize OMWW by transforming its constituents through various valorization strategies into high value-added commodities.

## 2. Olive oil

### 2.1. Production and consumption

Olive oil is widely consumed, with volumes rising steadily since 2005 (Aggoun et al., 2016). Olive farming and the olive oil industry are both economically and socially relevant, especially in the Mediterranean countries where about 98% of the world's olive oil is produced with an estimated production of above 15 M m<sup>3</sup>/yr (Ntougias et al., 2015). The most important olive oil producing country is Spain, followed by Italy, Greece, Turkey, Tunisia, Portugal, Morocco, and Algeria (Dourou et al., 2016). Outside the Mediterranean region, olives are cultivated in the Middle East, USA, Argentina, and Australia (Aparicio and Harwood, 2013).

In terms of demand, the consumption of olive oil in traditional markets is well known and deeply rooted in the populations' eating habits (Mili, 2006). Among traditional consumers of olive oil, Greece leads the ranking worldwide with an annual per capita consumption of 24 L, followed by Spain and Italy with 14 and 12 L, respectively (International Olive Council, 2017).

### 2.2. Types and designations

The olive oil from the olive tree *Olea Europaea* L. can be obtained solely by mechanical procedures or physical methods under certain conditions, especially thermal ones. These treatments do not lead to alterations to the oil and only include washing, decantation, centrifugation, and filtration (Dourou et al., 2016). One of the widely used procedures for olive oil extraction includes a traditional pressing method comprising a three-phase system that yields olive oil, a solid waste called olive pomace, and a liquid residue known as OMWW (Fig. 1).

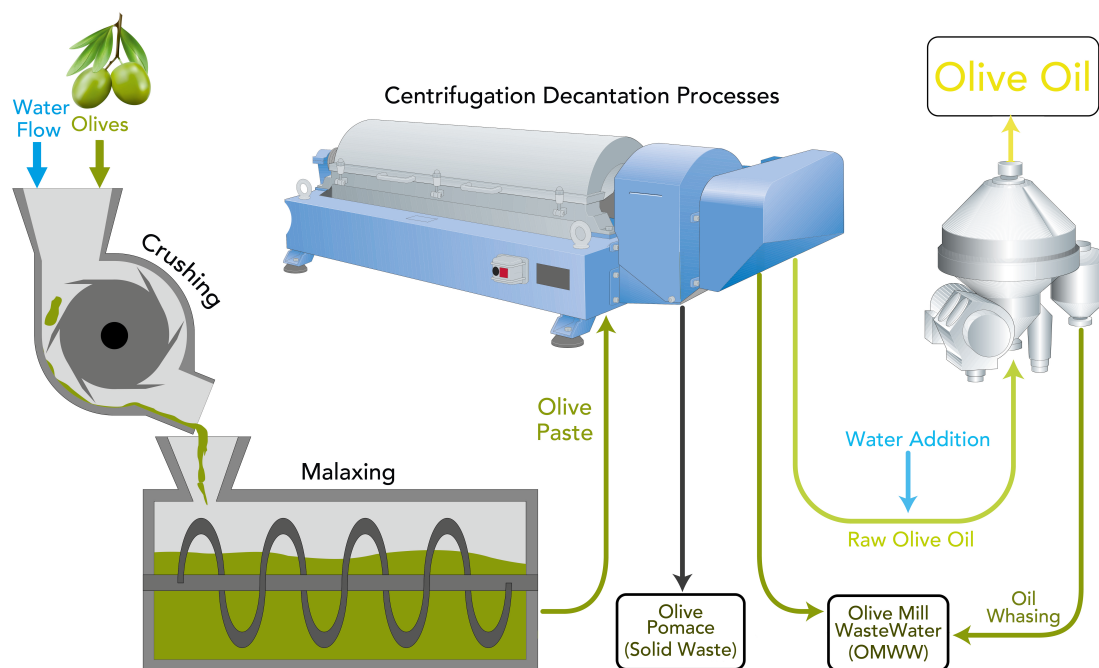


Fig. 1. Flowchart of obtaining olive oil through the three-phase procedure yielding three fractions: olive oil, olive pomace (solid residue), and OMWW.

Oil from olives are called virgin olive oil and, according to their acidity, the International Olive Council classifies them as:

- Extra virgin olive oil*: with maximum purity and low free acidity of up to 0.8 g oleic acid/100 g oil.
- Virgin olive oil*: with free acidity below or equal to 2 g oleic acid/100 g oil.
- Ordinary virgin olive oil*: with free acidity no higher than 3.3 g oleic acid/100 g oil.

### 3. Olive mill wastewater; main liquid waste stream of the olive oil industry

The type, quality, and quantity of residues originated during oil extraction or olive fruit treatment depend not only on variety, maturity of olives, and region of origin of the olive trees but also on the extractive technology used (Roig et al., 2006). The methods used for olive oil extraction include discontinuous (pressing) or continuous (centrifuging) processes with the former regarded as the oldest and most extensive system for processing olives in both traditional mills and modern industries. Although this method generates a small volume of OMWW (up to 60 L/100 kg olives), the wastewater has a higher COD compared to the OMWW produced by other processes (Di Giovacchino et al., 2002). As for the continuous olive oil extraction method, an industrial decanter is used to separate the phases obtained by centrifugation. There are two centrifugation systems, namely three-phase and two-phase systems that differ in the number of the end fractions generated (Roig et al., 2006). The continuous three-phase system produces a solid waste called olive husk or olive pomace and two liquid phases, i.e., oil and OMWW, while the two-phase technology separates the olive paste into two fractions: olive oil and a semi-solid residue known as two-phase olive-mill waste (TPOMW), a mixture of olive husk and OMWW. The continuous three-phase decanter presents some advantages like complete automation, better oil quality, and smaller area needed, however, it also presents some inconveniences such as expensive installation, greater energy consumption, and warm water addition, producing a larger quantity of OMWW (80–120 L/100 kg olives). In fact, the two-phase extraction process has been labeled as “ecological” because of reduced water consumption (Roig et al., 2006; Kapellakis et al., 2008).

Nevertheless, the resulting TPOMW has peculiar physico-chemical properties, it is generated in considerable quantities (10 L/100 kg olives), and is difficult to manage because its pollutants are more concentrated (Dermeche et al., 2013).

According to the International Olive Oil Council, pressing processes are usually used for olive oil extraction and significantly more or less water is required depending on the system employed (Therios, 2009). Even though traditional pressing is a relatively obsolete technology, it is still in use by various olive oil producers (Roig et al., 2006).

#### 3.1. Composition and characteristics

The characteristics of OMWW are variable depending on geographic location of olives, type and maturity, method of extraction, climatic conditions, cultivation/processing procedures, and process mode (batch or continuous) involved in obtaining oil, etc. (Fountoulakis et al., 2002; Paraskeva and Diamadopoulos, 2006). This effluent is harmful to sewage treatment plants due to the large amounts of organic and suspended matters, and especially because of its oil content (Rytwo et al., 2013). OMWW usually contains high CODs, BODs, as well as elevated amounts of total phenols, carbohydrates, polysaccharides, fatty acids, polyalcohols, pectins, and tannins (Lesage-Meesen et al., 2001). The typical brownish-black color of OMWW is ascribed to the presence of polymeric phenols. These substances represent a lignin-like structure and constitute the most resistant fraction of this waste stream (Hamdi, 1993). In addition to its high-polluting power, OMWW usually exhibits a high level of phytotoxic and antibacterial activity due to the presence of various phenolic compounds, making it difficult to treat using biological technologies and soil microbial communities (Karpouzias et al., 2010; Ouzounidou et al., 2012).

#### 3.2. Disposal and environment implications

OMWW is produced in huge quantities around the world annually, and its unsafe disposal leads to water, air, and land pollution in the proximity of olive oil processing units.

The large volumes of OMWW generated and the brief period of olive oil production, between November and March in the Mediterranean olives-growing countries, aggravate the environmental damage caused when these

effluents are disposed of into the environment without treatment (Aviani et al., 2012; Ntougias et al., 2013). Dumping of untreated OMWW causes severe environmental impairments ranging from altering the color of natural water sources and toxicity to aquatic life to contamination of surface and ground waters, soil quality adulteration, cytotoxicity, phytotoxicity, and nauseous odor (Dermeche et al., 2013).

OMWW discharge into soil has direct detrimental effects not only on plant growth and microbial metabolism but also on the physicochemical properties of the soil (Paredes et al., 1987). Soils could have differences in their intrinsic buffering capability depending on their origin and thus, may react differently to the same applied disturbance. However, it is important to highlight the fact that, due to the high concentration of recalcitrant compounds in OMWW, its direct use in fertigation, for instance, can inhibit plant seed germination. Moreover, oil compounds present in these streams may cause increased soil hydrophobicity and diminished water retention and infiltration rates (Kavvadias et al., 2010; Piotrowska et al., 2011). Although the impact of OMWW application on soil properties seems to be the result of contrary effects, in agreement with the balance between beneficial and toxic organic and inorganic compounds, the main conclusions of the research in this field confirm that the direct application of untreated OMWW has severe consequences in the long term. However, depending on the OMWW dilution rate and by controlling its application in soils, the above-mentioned negative effects of this wastewater could be reduced and its application could be beneficial (Magdich et al., 2012).

Several compounds dissolved in OMWW have influences on surface waters: 1) reduced sugars can stimulate microbial respiration lowering dissolved oxygen availability; 2) high concentrations of dark phenolics can alter the color of natural water resources (streams and rivers); and 3) lipids may form an impenetrable layer on the receiving water-surface blocking out sunlight and oxygen, thus inhibiting plant growth and favoring alga proliferation (Kapellakis et al., 2006). Therefore, the disposal of OMWW in sea-, river- or groundwater has resulted in serious concerns. It has been demonstrated that direct OMWW dumping into marine environments could cause pre-pathological alterations in marine organisms. The results obtained have shown the occurrence of structural deterioration in the aquatic populations due to the polluting effects of OMWW as well as decreases in water capability of reducing the impact through internal mechanisms of self-purification (Danellakis et al., 2011; Pavlidou et al., 2014). There are some cases where evaporation ponds or lagoons were built to contain this wastewater; however, they were rarely a suitable alternative to stabilize and safely accommodate this liquid waste. In most instances, the bottom of these ponds was permeable, thus leading to the introduction of OMWW into nearby systems such as agricultural soils and adjoining surface- and groundwater. In fact, it has been reported that OMWW spreading might increase phenolic compounds in soil and groundwater during the active period of olive factories (Koutsos et al., 2018).

OMWW can also contaminate the air if it is stored in open tanks or disposed of into large fields. It can undergo fermentation and emit methane and other pungent gases such as hydrogen sulfide, creating unpleasant odor pollution (Niaounakis and Halvadakis, 2006).

### 3.3. Policies and regulations

Pollution awareness and policies have played a minor role in finding uses for OMWW; nevertheless, there is a collective concern throughout the world with respect to environmental pollution caused by this kind of agro-industrial wastewaters. The United States Environmental Protection Agency (EPA) developed and implemented related policies and regulations more than a decade ago (Singh, 2006), while the European Commission established laws to influence the environmental regulations and technical aspects of industrial OMWW discharge in all olive oil-producing countries. In Spain, the government has prohibited the discharge of OMWW into receiving media and the two-phase olive oil extraction method (yielding olive wet pomace as the sole residue) is currently being used to decrease water requirements and consequently, the amounts of wastes generated (Azbar et al., 2004). In Italy, rules and regulations exist governing OMWW dumping in soils amenable to agricultural practices (Rana et al., 2003) while through a special permission obtained some time ago, Portugal allows land spreading of this wastewater (decree-law no. 236/98) (Cheng, 2006).

In Greek legislations, there are no specific guidelines about OMWW discharge. Moreover, laws concerning land applications and recycling of these

effluents are yet to be adopted (Azbar et al., 2004; Kapellakis et al., 2006).

Other olive producing countries lack wastewater disposition policies but employ sustainable practices with this respect. For instance, in Tunisia, OMWW produced every year is collected and poured into large concrete evaporation reservoirs adjacent to wastewater treatment plants (Ammar and Ben Rouina, 1999). In Turkey, olive oil industries are generally located in the west and south of the territory and the main obstacle for safe OMWW disposal is that factories are small and scattered throughout a large geographical area. The Turkish government is yet to specifically regulate the release of OMWW (Yay et al., 2012).

The European wastewater policy necessitates that only OMWW treated in accordance with relevant quality standards and the provisions required by Directive on Urban Wastewater Treatment (1991) (Directive 271/91, later amended by Directive 15/98) could be discharged into receiving waters bodies.

### 4. Physicochemical treatments of OMWW

The most relevant physicochemical treatments of OMWW include methods such as evaporation, reverse osmosis, ultrafiltration, coagulation, oxidation, thermal drying, and advanced oxidation technologies such as ozonation, Fenton processes, and electrochemical oxidization (Mert et al., 2010; Scoma et al., 2011).

Although several physicochemical processes are used for OMWW treatment, these are not completely successful. Their implementation is often associated with large-scale feasibility and cost-efficiency issues which may be further accompanied by other technical or environmental problems related to emission of air pollutants, membrane fouling, toxicity induced by radical species, and the formation of large quantities of toxic sludge, among others.

### 5. Biological treatments of OMWW

Among the biological treatments of OMWW, anaerobic digestion has been proposed as a promising technology for olive residues management to produce energy (biogas). However, numerous obstacles still need to be overcome such as growth inhibition of methanogenic archaea by phenolic compounds, low pH, low nitrogen concentration, etc. (Orive et al., 2016). Other biological alternatives are aerobic methods and include composting as well as treatments with fungi, bacteria, and algae (Pinto et al., 2003; Dhouib et al., 2006; Tziotziou et al., 2007; Chowdhury et al., 2013). The success of each method depends on the technology used. Nonetheless, it should be noted that any biological treatment alternatives should constitute a sustainable strategy for the management of OMWW, i.e., through the exploitation of the nutritive potential of this waste stream for the production of various high added-value compounds.

### 6. Valorization and biotransformation of OMWW into high added-value compounds

OMWW can be considered as a resource to be recycled and recovered. Present-day strategies aim at cleaner production methods or practices that consider not only olives and olive oil economic production but also the potentially beneficial uses of their residues. The bio-based exploitation alternatives for OMWW to obtain added-value compounds are summarized in Figure 2.

#### 6.1. Uses of OMWW in renewable energy and biofuels industry

It is obvious that the need to reduce dependence on conventional fossil fuels in favor of new alternative energy resources is a top global priority. Green energies could effectively contribute to mitigation of greenhouse gases emissions and their consequent unfavorable impacts including global warming and climate change (Hill, 2009). In this sense, OMWW is a promising raw material for bioenergy and biofuel production owing to its low to moderate contents of nitrogen, sugars, volatile acids, polyalcohols, and fats (Dermeche et al., 2013).



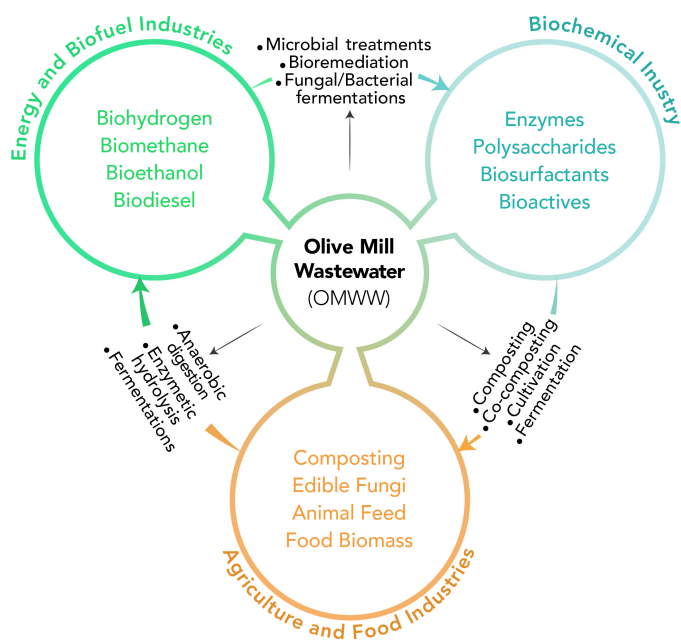


Fig. 2. Alternatives for microbial valorization and potential uses of OMWW.

#### 6.1.1. Biohydrogen

Biohydrogen (bio-H<sub>2</sub>) is considered as a renewable energy carrier with the capacity to supply the forthcoming energy demands and with the potential to replace fossil fuels. That is why it is acknowledged as the versatile fuel of the

future (Kotay and Das, 2008). Bio-H<sub>2</sub> production involves a wide range of reactions, including direct and indirect biophotolysis, photo-fermentation, and dark-fermentation. Moreover, bio-H<sub>2</sub> can be produced by a large number of microorganisms (mainly bacteria) with distinctive physiological and metabolic features through single or combined catabolic pathways (Table 1). Certain microbial production processes involve the use of photosynthetic products by enzymes with either hydrogenase or nitrogenase activity as bio-H<sub>2</sub>-producing proteins (Kotay and Das, 2008). The low nitrogen content of OMWW makes it a favorable substrate for photo-fermentative bio-H<sub>2</sub> production because high NH<sub>4</sub><sup>+</sup> concentrations inhibit nitrogenase synthesis and activity (Uyar et al., 2012). However, numerous investigations stress on the fact that bio-H<sub>2</sub> production by photosynthetic bacteria using OMWW as a substrate necessarily requires counteracting its inhibitory effects caused by the dark color of this wastewater. In order to overcome this problem, high dilution rates of OMWW has been proposed. Eroglu et al. (2004) studied bio-H<sub>2</sub> production from diluted OMWW within the range of 1% to 20% (v/v) using the photosynthetic bacterium *Rhodobacter sphaeroides* in column photobioreactors. With this agro-industrial wastewater as the sole substrate source, the authors reached a maximum bio-H<sub>2</sub> production potential (HPP) of around 13.9 L/L when the 2% OMWW-containing media was used. Later, the same researchers performed a comparative study with different samples of OMWW (diluted to 4% v/v) from different olive oil factories in Anatolia and Turkey, and investigated bio-H<sub>2</sub> production under anaerobic photo-fermentative conditions using *R. sphaeroides* O.U.001. They found a linear relationship between C/N molar ratios and bio-H<sub>2</sub> production capacities. Maximum HPP (20 L/L of OMWW) was recorded from the OMWW samples with the greatest organic content (mostly acetic, aspartic, and glutamic acids) and the highest C/N molar ratio (Eroglu et al., 2009).

*R. sphaeroides* is the most investigated photo-fermentative bacterial species in bio-H<sub>2</sub> production coupled with OMWW treatment. Eroglu et al. (2011) studies bio-H<sub>2</sub> production linked to OMWW photodegradation by *R. sphaeroides* O.U.001 strain using diluted cultures of this wastewater (2% v/v) supplemented with iron and molybdenum. These two metals were selected because both are part of the nitrogenase enzyme complex involved in photosynthetic processes. The diluted iron-supplemented OMWW-based cultures not only showed a significantly increased production (125 mL bio-H<sub>2</sub> vs. 62 mL obtained in the presence of molybdenum) but also yielded

Table 1.  
General reactions of bio-hydrogen (bio-H<sub>2</sub>) production and main participating microorganisms. Modified from Dermeche et al. (2013).

Variety of bio-hydrogen (bio-H <sub>2</sub> ) production pathways	Microorganisms	References
<i>Bio-H<sub>2</sub> production reactions in a single way</i>		
Bio-H <sub>2</sub> production by dark fermentation $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ $C_6H_{12}O_6 \rightarrow CH_3CH_2COOH + CH_3COOH + CO_2 + H_2$ $C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$	Heterotrophic bacteria ( <i>Clostridium</i> sp.)	Lin et al. (2007); Kotay and Das (2008)
Bio-H <sub>2</sub> production by direct/indirect biophotolysis $12H_2O \rightarrow 12H_2 + 6O_2$	Green micro algae ( <i>Chlamydomonas reinhardtii</i> ) Cyanobacteria ( <i>Anabaena</i> sp.)	Kotay and Das (2008)
Bio-H <sub>2</sub> production by photo-fermentation (photo decomposition of organic compounds) $C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2$	Purple-phototropic (anaerobes photosynthetic) bacteria ( <i>Rhodobacter sphaeroides</i> , <i>Rhodospseudomonas palustris</i> )	Kataoka et al. (1997); Mahyudin et al. (1997); Tanisho et al. (1998)
Other single reaction of bio-H <sub>2</sub> production (from CO) $CO + H_2O \rightarrow H_2 + CO_2$	Photosynthetic bacteria	Kotay and Das (2008); Ena et al. (2010); Eroglu et al. (2010)
<i>Bio-H<sub>2</sub> production through combined ways</i>		
First Stage: Dark fermentation $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$	Facultative anaerobes bacteria	Kotay and Das (2008); Eroglu et al. (2006)
Second Stage: Photo-fermentation $2CH_3COOH + 4H_2O \rightarrow +4CO_2 + 8H_2$	Photosynthetic bacteria ( <i>Rhodobacter sphaeroides</i> )	

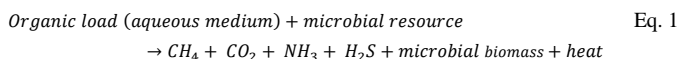
better wastewater treatment results by removing 48.1% of the initial COD value compared with the control reactor in which the maximum COD removal efficiency achieved was 30.2%.

Ena et al. (2010) evaluated the purple bacteria *Rhodospseudomonas palustris* (strain 6A) for bio-H<sub>2</sub> production using cylindrical (CPBR) and flat (FPBR) photobioreactors in both batch and semi-continuous conditions, under continuous light at 30 °C. The bacterium was grown in a dry-Azolla and activated carbon pretreated OMWW (25% v/v) and removed 80% COD and 98% phenols. Successful bio-H<sub>2</sub> production results were also obtained throughout the experiments. More specifically, during the lag-phase of growth, no hydrogen was produced in none of the photobioreactors; however, bio-H<sub>2</sub> generation began at 406 h of culture, and final volumes of 0.53 L in the CPBR and 0.31 L in the FPBR were recorded in the batch regime. When this operation mode was compared with the semi-continuous mode, the latter showed a better performance with significantly higher bio-H<sub>2</sub> production.

Other microorganisms have also been studied in photo-fermentative processes for bio-H<sub>2</sub> production employing OMWW as a substrate. Faraloni et al. (2011) proposed a two-stage fermentation for bio-H<sub>2</sub> photoproduction by the green microalga *Chlamydomonas reinhardtii* cultivated in OMWW diluted and pretreated by biofiltration with *Azolla caroliniana* and granular activated carbon as remediating agents. They observed that under sulfur-deprived conditions, *C. reinhardtii* metabolism was able to shift to bio-H<sub>2</sub> production through the photosystem II-driven water splitting of this microalga as well as the fermentation of accumulated carbohydrates.

### 6.1.2. Biomethane

Effluents with high organic load concentrations can also be converted into methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) in anaerobic digesters through the concerted actions of different microbial populations. The whole methanogenesis reaction may be summarized as follows (Eq. 1)



Usually, methane production from OMWW involves aerobic or anaerobic pretreatment of this wastewater followed by a two-phase anaerobic digestion process. Fungi proved to be excellent candidates to pretreat OMWW prior to anaerobic digestion. Hamdi (1991) pretreated OMWW with *Aspergillus niger* and the amount of CH<sub>4</sub> produced was twice higher in the subsequent anaerobic digestion. Borja et al. (1995) carried out a comparative kinetic study of the OMWW anaerobic digestion without pretreatment and pretreated by the fungus *Aspergillus terreus*. The results obtained demonstrated that the pretreatment applied influenced methane volumetric production, resulting in 1.1 L and 0.6 L CH<sub>4</sub>/L<sub>OMWW</sub>/d for the reactors processing predigested and untreated OMWW, respectively. The aerobically pre-fermented effluent drastically reduced the levels of both phenolic compounds and biotoxicity while significantly increased CH<sub>4</sub> production by up to 83%.

During the first phase of the two-phase anaerobic digestion, macromolecules such as carbohydrates, proteins, and lipids are transformed by hydrolytic and acidogenic fermentative bacteria into simple organic compounds (sugars, volatile fatty acids, and amino acids) and intermediates such as volatile organic acids (mainly acetic, propionic, and butyric), alcohols (mainly ethanol), ketones (ketone), CO<sub>2</sub>, and hydrogen. In the second phase, through the interactions between methanogenic and acetogenic microorganisms, all these metabolites are metabolized and transformed into CH<sub>4</sub> and CO<sub>2</sub> (Moraes et al., 2015). Fezzani and Ben Cheikh (2010) carried out the two-phase anaerobic digestion of OMWW together with an olive mill solid waste using five semi-continuous digesters at mesophilic temperature (37 ± 2 °C). In the acidifier-stage (first step), two of the five reactors were operated using a hydraulic retention time (HRT) ranging between 14 and 24 d, and an organic loading rate (OLR) ranging from 5.54 to 14 g COD/L/d. The methanogenesis stage (second step) was conducted in the three remaining digesters at 18, 24 and 36 d of HRT, with OLRs ranging from 2.28 to 9.17 g COD/L/d. The results indicated that the volatile fatty acids (VFA) content was augmented by increasing HRT or feed concentration and the maximum values were obtained at the HRT of 24 d, which corresponded to an OLR of 8.17 g COD/L/d. Their two-phase anaerobic digestion system led to the best methane productivity (32 L/L<sub>OMWW</sub>) compared to those achieved with conventional one-phase anaerobic digesters (Fezzani and Ben Cheikh, 2007). This could be ascribed to the fact that the high levels

of VFAs produced during the acidification step could be easily metabolized and biotransformed into CH<sub>4</sub> and CO<sub>2</sub> in the subsequent step.

Some researchers have studied other OMWW pretreatment alternatives prior to anaerobic digestion for biomethane production. Azbar et al. (2008) demonstrated that chemical pretreatment of OMWW by employing acids, followed by a coagulation-flocculation process using Al<sub>2</sub>SO<sub>4</sub>, FeSO<sub>4</sub>, and FeCl<sub>3</sub>, enhanced the anaerobic biodegradability of this agro-industrial residue leading to 80% higher CH<sub>4</sub> production compared with the untreated effluent.

OMWW co-digestion with other wastes such as poultry manure, slaughterhouse wastewaters, winery residues, and liquid cow manure has also been investigated, resulting in CH<sub>4</sub> yields of more than 250 L/kg COD (Gelegenis et al., 2007; Fountoulakis et al., 2008; Dareioti et al., 2010).

### 6.1.3. Bioethanol and biodiesel

The huge amounts of organic matters in OMWW also make it a suitable feedstock for ethanol and biodiesel production. However, it is necessary to remove or reduce its phenolic compounds in order to use its carbohydrate and lipid fractions to produce these biofuels. Massadeh and Modallal (2008) investigated the capability of a *Pleurotus sajor-caju* strain to degrade phenols in OMWW preconditioned by different treatments: 50% water-diluted and undiluted OMWW thermally processed at 100 °C, and OMWW thermally processed and predigested with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Results showed that the degradation of phenols with *P. sajor-caju* reached as high as 50% for the thermally processed diluted OMWW, 53% for the thermally processed OMWW pretreated with H<sub>2</sub>O<sub>2</sub>, and 58% for the thermally processed undiluted OMWW. The impact of this biological pretreatment was subsequently tested with the yeast *Saccharomyces cerevisiae*. According to the results obtained, pretreatment with *P. sajor-caju* led to enhanced ethanol production from OMWW. The highest ethanol yield of 14.2 g/L was obtained after 48 h of fermentation using 50% diluted and thermally processed OMWW.

Sarris et al. (2014) also evaluated *S. cerevisiae* MAK-1 for simultaneous OMWW remediation and production of added-value compounds. Under aerated conditions in non-sterile shake-flask cultures, cultures in molasses-based media were supplemented with OMWW. No significant decreases in ethanol (34.3 g/L) and biomass production (7.3 g/L) were observed in comparison with the control experiments (i.e., cultures with no OMWW supplementation). Under similar but aerated bioreactor conditions, biomass production decreased (5.7 g/L) while on the contrary; ethanol yield was notably enhanced (up to 41.8 g/L).

Some studies have also reported on the exploitation of olive oil liquid and solid by-products and their bioconversion to biodiesel. Yousef et al. (2010) argued that *Lipomyces starkeyi* could be a promising yeast strain as a non-conventional source of oil and used it for the conversion of OMWW into lipids for biodiesel production. They demonstrated that the investigated yeast was capable of proliferating in the presence of undiluted OMWW, with no external organic compound supplementation requirements, and significant reduced both total organic carbon and total phenols contents of the wastewater. Nevertheless, when the OMWW was 50% diluted by distilled water, polluting compounds were reduced and a significant increase in lipid yield was observed (28.6% vs. 22.4% in the undiluted OMWW). The fatty acid profiles obtained showed a prevalence of oleic acid, demonstrating the potential of this yeast to store lipids suitable for (second-generation) biodiesel production.

Sarris et al. (2017) evaluated the cultivation of *Yarrowia lipolytica* on commercial glucose-supplemented media plus OMWW in high concentrations (i.e., high phenolic compounds quantities). Their results revealed the capability of the strain to grow and produce highly lipid enriched biomass and citric acid despite the high concentration of phenolic compounds present. Satisfactory citric acid quantities were produced in nitrogen-limited media while non-negligible biomass production was observed in carbon-limited media. The addition of OMWW in the medium favored the accumulation of storage lipids suggesting that OMWW seemed to be a "lipogenic" substrate. Both nitrogen and carbon-limited fermentations resulted in a remarkable discoloration and a non-negligible reduction of phenolic compounds in the media. In another investigation, *L. starkeyi* NRRL Y-11557 and *Y. lipolytica* strains also showed a noteworthy ability to accumulate lipids (15–25%, w/w) when cultivated on OMWW-

based media. Oleic acid and palmitic acid were the main fatty acids produced when the culture medium was enriched with OMWW as low-cost carbon source (Dorou et al., 2016).

Bellou et al. (2014) demonstrate the ability of Zygomycetes strains to grow on OMWW and bioconvert it into lipids containing polyunsaturated fatty acids in submerged cultures; while in parallel, phenolic compounds were also removed. In liquid media containing OMWW as the sole carbon source, the maximum cell mass produced, the maximum specific growth rate, as well as cell mass and lipid yields (60% w/w) were almost unaffected in comparison with the control sample. Oleic and palmitic acids were the predominant fatty acids determined. Gamma-linolenic acid was found in high percentages (up to 17.7%, w/w) in the lipid produced by *Zygorhynchus moelleri*. Similarly, adaptation of *Y. lipolytica* in OMWW-based media favored the biosynthesis of cellular unsaturated fatty acids, principally oleic and palmitoleic acids (Papanikolaou et al., 2008).

## 6.2. Uses of OMWW in the biochemical industry

The production of biochemicals by various microorganisms has a long history and continues to be one of the most interesting biotechnological alternatives to valorize OMWW. The synthesis of bio-based compounds from OMWW is doubly beneficial since it not only valorizes this low-cost waste stream as feedstock but also reduces the environmental damages caused by its natural discharge.

### 6.2.1. Enzymes

The biotechnological application of microbial enzymes has grown considerably over the last 20 years, especially with respect to the biotransformation of agro-industrial wastes (Gassara et al., 2010). Fungi are well-known microorganisms due to their ability to synthesize a wide range of biological catalysts that can be used in various industrial processes. Specifically, ligninolytic fungi constitute a powerful tool for OMWW degradation and detoxification since they have demonstrated their ability to remove recalcitrant compounds like the ones present in these effluents through the production of non-specific oxidative enzymes such as phenoloxidases, polyphenoloxidases, and peroxidases (Ntougias et al., 2013 and 2015). Table 2 shows examples of the production of ligninolytic enzymes coupled with OMWW bioremediation under several culture parameters and using different ligninolytic fungi.

Fenice et al. (2003) used an OMWW-based medium (2-fold diluted OMWW supplemented with 0.5% sucrose and 0.1% yeast extract) for the growth of *Panus trigrinus* CBS 577.79 with the consequent production of laccase (Lacc) and Manganese peroxidase (MnP). The highest activity levels achieved were  $4600 \pm 98$  U/L and  $370 \pm 15$  U/L, respectively, in a stirred-tank reactor and  $4300 \pm 23$  U/L and  $410 \pm 22$  U/L, respectively, in an airlift reactor.

Koutrotsios et al. (2016) evaluated the suitability of OMWW (12.5%, 25%, and 50% v/v) as a substrate for the production of Lacc and MnP by the fungus *Hericium erinaceus*. During OMWW bio-treatment, the enzymes profiles obtained revealed a maximum Lacc activity of 134 U/L on 28 d in 50% OMWW. Although to a lesser extent, MnP was also excreted during the first week of treatment and peaked during the second week for two out of the three treatments (more than 20 U/L and  $\geq 10$  U/L in OMWW 12.5% and 50%, respectively). Manganese-independent peroxidase (MIP) was also generated toward the end of growth, but with a low activity in the lowest wastewater concentration ( $\sim 15$  U/L in 12.5% OMWW).

In a study performed by Zerva et al. (2017), two ligninolytic white-root fungal strains, *Pleurotus citrinopileatus* LGAM 28684 and *Irpex lacteus* LGAM 238, were tested for their OMWW oxidative capacity. The treatment of OMWW (25% v/v) was investigated under several culture conditions, namely different pH, agitation speed, as well as nitrogen-based supplements and their concentration. The selected parameters were pH 6, agitation rate 150 rpm, 30 g/L corn steep liquor as a nitrogen source for *P. citrinopileatus* and 20 g/L diammonium tartrate for *I. lacteus*. Employing OMWW as substrate, the production of biotechnologically valuable enzymes such as Lacc ( $1048.9 \pm 2.9$  U/L for *P. citrinopileatus* and  $57.4 \pm 2.2$  U/L for *I. lacteus*), MnP ( $303.7 \pm 15.2$  U/L for *P. citrinopileatus* and  $100.2 \pm 5.0$  U/L for *I. lacteus*), and MIP ( $735.0 \pm 4.27$  U/L for *P. citrinopileatus* and  $674.9 \pm 33.0$  U/L for *I. lacteus*) was demonstrated with simultaneous effluent degradation and detoxification (above 90% color and phenols reduction within a 24 d cultivation period).

Other enzymes such as lipases and pectinases can also be obtained through OMWW fungal treatment. Cordova et al. (1999) obtained lipases for application in the dairy, pharmaceutical, detergent, and other industries from OMWW fermentation based on the (variable) amounts of remnant oil present in this residue. Although some filamentous fungi have the ability to synthesize lipase enzymes such as *Aspergillus oryzae*, *Aspergillus niger*, *Geotrichum candidum*, *Penicillium citrinum*, *Rhizopus arrhizus* and *Rizhopus oryzae*, and have been consequently recognized as lipolytic species (Crognale et al., 2006), D'Annibale et al. (2006) reported *Candida cylindracea* NRRL Y-17506 as a promising yeast with high potentials for lipase production from OMWW supplemented with  $\text{NH}_4\text{Cl}$  and olive oil (optimal lipase activity of 9.23 U/cm<sup>3</sup>).

OMWW pretreated with precipitating agents and supplemented with a pectin-rich residue (sunflower by-product) was found to be a good medium for the production of pectolytic enzymes by the yeast strain *Cryptococcus albidus* var. *albidus* IMAT 473. The enzyme obtained was compared with commercially available preparations (Pectinex by Novo Enzymes, Pectinase by Fluka, and Ultrazym by Ciba-Geigy), and was shown to be an endopolygalacturonase with a large spectrum of activity on pectin with different degrees of methylation (Federici, 1985; Federici et al., 1988; Petruccioli et al., 1988).

### 6.2.2. Polysaccharides

Similar to the other agro-industrial residues, OMWW could be a strong potential source for the production of biomolecules with industrial applications, exopolysaccharides (EPSs) not being the exception. Although at present most of these EPSs come from plants and algae (with the exception of xanthan and curdlan), the use of inexpensive raw materials to produce EPSs by microorganisms represents a challenge since not only the properties of these compounds could be similar to or almost identical with those derived from vegetable biomass, but also because they could reduce production costs (Sutherland, 1998).

EPSs are bio-based extracellular and polymeric substances with diverse structural complexities and biological functions, and have extremely versatile applications. They can be used in the food, pharmaceuticals, cosmetics, and bioremediation fields (Liang and Wang, 2015).

Xanthan is one of the most commercially added-value microbial EPS. It is a polymer of repeated units of glucose, mannose, and glucuronic acid at a 2:2:1 ratio. Currently, xanthan is widely used in cosmetic formulations as an emulsion stabilizer or as a food supplement and a rheology modifier; it is also a thickening agent in salad dressings (Petri, 2015). The first xanthan production from OMWW was described by López and Ramos-Cormenzana (1996). Employing a *Xanthomonas campestris* NRRL B1459-S4L41 strain, they demonstrated that the maximum xanthan production of up to 4 g/L could be achieved when the bacterium was grown in 30% v/v OMWW solution. Moreover, EPS production was increased when phosphate-buffer was supplemented to the medium, reaching a final xanthan concentration of 6.3 g/L. In a different experiment, four *X. campestris* strains were tested in different % v/v OMWW solutions. Highest xanthan concentrations were obtained when using 30% v/v OMWW, with values ranging from 3.48 to 7.01 g/L (López et al., 2001).

Other EPS-producing bacteria capable of growing in OMWW have also been reported. Aguilar et al. (2001) reported the synthesis of a heteropolysaccharide consisting of fucose, xylose, rhamnose, arabinose, mannose, galactose, and glucose units, by using *Paenibacillus jamilae* sp. and raw OMWW. Ruiz-Bravo et al. (2001) also reported the production of an EPS when *P. jamilae* CP-7 was grown on an 80% v/v OMWW-based medium, and Morillo et al. (2007) obtained an EPS composed of glucose, galactose, mannose, arabinose, rhamnose, hexosamines, and uronic acid, also from diluted 80% v/v OMWW by using *P. jamilae* CECT 5266. Aguilar et al. (2008) isolated 60 different strains of the genus *Paenibacillus* from compost irrigated with OMWW. The screening-trials, performed in shake flasks, showed that only ten strains were able to synthesize EPSs. Out of those, *P. jamilae* CP-38 was the strain with the best yield (4.2 g/L of EPS in 80% v/v OMWW), within 48 h. Subsequent experiments using a 2 L bioreactor demonstrated an increase in the amount of the EPS produced, reaching 5.2 g/L at the end of 72 h of fermentation.

Fungi are also known for their ability to synthesize EPSs. Ramos-Cormenzana et al. (1995) reported the growth and pullulan synthesis by

**Table 2.**  
Main enzymes produced during the biological treatment of OMWW by ligninolytic fungi.

Microorganism	Treatment conditions	Reduction (%)			Time	Enzymes	References
		Color	Phenolic	COD/TOC			
<i>Lentinus edodes</i> (SC-495)	Five-fold diluted OMWW in medium containing glucose 5 g/L and yeast extract 2 g/L. Trials were performed in shaking-flasks with 5% (v/v) inoculum.	90	66	85	12 d	Phenoloxidases MnP	Vinciguerra et al. (1995)
<i>Pleurotus ostreatus</i>	10% (v/v) water-diluted OMWW was incubated with the fungus previously adapted in PDYm medium (potato dextrose broth, yeast extract, and maltose) containing up to 20% OMWW.	n.a.	90	n.a.	100 h	Phenoloxidases	Martirani et al. (1996)
<i>Abortiporus biennis</i> (CCBAS 521)		9.10	54.5				
<i>Pleurotus ostreatus</i> (CCBAS 472)	Prior to biological treatments, OMWW was 50% (v/v) water-diluted and the pH was adjusted to 6.0 with H <sub>3</sub> PO <sub>4</sub> . The white-rot fungi were first cultivated on 50% water-diluted OMWW plus agar 1.6% (w/v).	48.9	51.5	n.a.	30 d	Lacc MnP	Aggelis et al. (2002)
<i>Panellus stipticus</i> (CCBAS 450)		8.40	42.2			MnP (only observed in <i>P. ostreatus</i> and <i>A. biennis</i> )	
<i>Dichomitus squalens</i> (CCBAS 751)		n.a.	36.4				
<i>Pleurotus flavido-alba</i>	Bioreactor was filled with basal medium plus veratryl alcohol (0.43 g/L), Tween 20 (0.5 g/L), and supplemented with Mn(II). After 5 d of fermentation, concentrated and sterilized OMWW was added to the bioreactor.	70	51	n.a.	14 d	MnP Lacc	Blázquez et al. (2002)
<i>Pleurotus ostreatus</i>	Undiluted OMWW thermally processed.		65	9	21 d	MnP	Fountoulakis et al. (2002)
	OMWW thermally processed (at 100 °C) and water diluted at 50% (v/v).	n.a.	67	12	19 d	LiP	
	50% (v/v) water-diluted and sterilized (120°C, 1 atm) OMWW.		78	10	21 d	Lacc	
<i>Pleurotus</i> spp. LGAM P105			69				
<i>Pleurotus</i> spp. LGAM P112	Media containing 75% OMWW and 25% distilled water, without any addition of nutrients or pretreatment.	n.a.	71	n.a.	24 d	Lacc	Tsioulpas et al. (2002)
<i>Pleurotus</i> spp. LGAM P113			73				
<i>Pleurotus</i> spp. LGAM P116			73				
<i>Pleurotus sajor-cajé</i>		MWL50: 72.1 MWL75: 54.5	n.a.	49.9 36.7		MnP Lacc	
<i>Lentinus (Lentinula) tigrinus</i>	Discoloration and COD removal from crude water-diluted OMWW at 50 g/L (MWL50) and 75 g/L COD (MWL75), without any additional carbon sources.	MWL50: 49.6 MWL75: 24.1	n.a.	39.2 24.1	20 d	MnP Lacc	Jaouani et al. (2003)
<i>Coriopsis polyzona</i>		MWL50: 74.6 MWL75: 33.0	n.a.	41.4 22.5		MnP Lacc LiP	
<i>Pycnoporus coccineus</i>		MWL50: 57.6 MWL75: 36.7	n.a.	47.1 25.1		MnP Lacc	

COD : Chemical Oxygen Demand; TOC :Total Organic Carbon  
Lacc : Lacasse; LiP : Lignin Peroxidase; MnP : Manganese (Mn) Peroxidase; MIP : Mn-independent Peroxidase  
n.a. : no available

*Aureobasidium pullulans* in 70% v/v OMWW solution. *Botryosphaeria rhodina* was investigated in different types of undiluted OMWW (i.e., OMWW<sub>1</sub>, obtained from a traditional three-phase extraction system; OMWW<sub>2</sub> and OMWW<sub>3</sub>, obtained from three-phase systems with low water consumption and OMWW<sub>4</sub>, obtained from a press extraction system) to produce β-glucan. Remarkable quantities of EPS were produced, although maximal β-glucan production was obtained at 120 h in OMWW<sub>4</sub> (17.2g/dm<sup>3</sup>) whose highest concentrations of COD and total sugars markedly promoted EPS production (Crognale et al., 2003). Later, the same researchers evaluated the technical feasibility of scaling up the EPS production process in a 3 dm<sup>3</sup> bench-top

reactor by *B. rhodina* cultured in OMWW<sub>4</sub>. Maximum production of β-glucan was slightly lower (16.8g/dm<sup>3</sup>) than the one obtained in shaking cultures, but a peak was achieved 24 h earlier (Crognale et al., 2006).

### 6.2.3. Biosurfactants

Biosurfactants are surface-active biological derivatives that, although produced mainly through fermentation employing microorganisms, are not directly associated with their growth, so that they are considered as secondary metabolites (Kourmentza et al., 2017). These compounds are



stable at extreme pH, salinity, and temperature conditions, and can be employed in novel applications such soil remediation, recovery of heavy metals, food or medicine (Shekhar et al., 2015).

Biosurfactants, amphipathic agents with hydrophilic “heads” and hydrophobic “tails”, are characterized by their ability to alleviate the surface tension of water or interfacial tension between two opposite phases, aqueous and hydrophobic. Due to residual oils and the presence of polysaccharides (Aguilera et al., 2008; Dermeche, 2013), OMWW constitutes a suitable carbon source for the production of biosurfactants in the form of rhamnolipids (glycolipid biosurfactants) or surfactins (lipopeptide biosurfactants). Mercadé et al. (1993) were the first to investigate the synthesis of rhamnolipids using *Pseudomonas* sp. JAMM and OMWW as the sole carbon source. After 72 h of incubation, rhamnolipid conversion yield reached 0.058 g/g<sub>OMWW</sub>, coinciding at the same time with 50% COD reduction and 55% removal of the total phenolic content in OMWW. Nevertheless, maximum rhamnolipid production was estimated at 14 g/Kg<sub>OMWW</sub>, after 150 h of fermentation. Two decades later, Colak and Kahraman (2013) cultured a wild-type strain and a recombinant strain of *P. aeruginosa* ATCC 10145 in OMWW for rhamnolipid production. With both wild-type and recombinant strains, the maximum rhamnolipid yield reached as high as 0.4 g/L after a growth period at 37 °C, at 100 rpm and 72 h. Ramírez et al. (2015) looked into rhamnolipid and surfactin synthesis with *P. aeruginosa* and *B. subtilis* strains, using 2-10% v/v OMWW solutions. The results obtained showed that surfactin reached production levels of 3.12 mg/L for *B. subtilis* in 2% OMWW that then dropped to 0.57 mg/L at the most often used concentrated OMWW solution (10% v/v). In contrast, *P. aeruginosa* reached rhamnolipid values from 8.78 in 2% OMWW to 191.46 mg/L when the highest OMWW concentration was used.

#### 6.2.4. Bioactive compounds

In nature, phenolics appear as single molecules (acid phenols), or highly polymerized compounds such as tannins. Nevertheless, the most common phenolics are those conjugated with mono-, di-, or oligosaccharides sugars, organic acids, and lipids, or even with alternative phenols coupled to hydroxyl radicals or, less frequently, to aromatic carbon atoms (Bravo, 1998; Shahidi, 2003).

OMWW has been widely investigated as a source of bioactive compounds, and the antioxidant activity of phenolics extracted from olive by-products has also been reported (Salido et al., 2015; Gullón et al., 2018). Phenols are present in OMWW mainly as colored pigments; their concentrations vary according to their chemical polarity, olive variety, and way of cultivation, as well as oil extraction methods and treatments applied to olive mill wastes (Dourou et al., 2016). Moreover, other factors including ripeness of the fruit, climate, agronomic conditions, storage conditions prior to oil extraction, and processing techniques could also have significant quantitative and qualitative impacts on OMWW bioactive compounds (Allouche et al., 2004; Obied et al., 2005).

Although the phenolic content may differ in OMWW, the main compounds described in the literature are phenolic acids, secoiridoids, and flavonoids (Lafka et al., 2011). Bianco et al. (2003) identified 20 prevalent classes of bioactive compounds in OMWW using HPLC-MS/MS, including phenyl-alcohols, phenols acids, secoiridoid derivatives, flavonoids (luteolin, luteolin-7-glucoside), and lignans. Visioli et al. (2002) reported oleuropein (an ester of elenolic acid) and hydroxytyrosol as primary bioactive compounds in OMWW. Other phenols also detected in OMWW are M4-methylcatechol, 4-hydroxybenzoic acid, protocatechuic acid, vanillic acid, 3,4-dihydroxyphenylglycol, homovanillic alcohol, 4-hydroxy-3,5-dimethoxybenzoic acid, 3,4-dihydroxyphenylacetic acid, 2-(4-hydroxy-3-methoxy)phenylethanol, and 2-(3,4-dihydroxyphenyl)-1,2-ethandiol (Capasso et al., 1992; Aramendia et al., 1996; Della Greca et al., 2001). All these compounds showed anti-hypertensive, anti-inflammatory, hypoglycaemic, and hypocholesterolemic properties, and in some cases, exhibited antimicrobial properties against certain bacteria, fungi, and mycoplasma (Ghanbari et al., 2012).

Bioconversion has been used to recover bioactive compounds from olive by-products. Khoufi et al. (2011) showed that an enzymatic extract from *Aspergillus niger*, mainly comprising  $\beta$ -glucosidase and esterase, had the potential to produce free simple phenolic compounds. The hydrolytic activity of the preparation was assayed upon three substrates: raw OMWW, the phenolic fraction extracted from OMWW with ethyl acetate, and its corresponding spent fraction. Huge quantities of bioactive phenolics

(especially hydroxytyrosol, HT) were released from the spent fraction (0.75 g/L) and from raw OMWW (0.56 g/L) after enzymatic treatment, with promising applications in food processing and pharmaceutical industries. Hamza et al. (2012) performed a similar study, treating OMWW enzymatically with  $\beta$ -glucosidase-rich *Aspergillus niger*, *Trichoderma atroviride*, and *Trametes trogii* culture broths in order to release compounds with antioxidants properties. In the first two cases, the amount of HT released increased from 0.05 g/L up to 1.1 g/L and 0.50 g/L, respectively, while *T. trogii* broth culture led to the oxidation of the phenolics instead of their recovery because of the high Lacc enzyme titers recorded for this fungus.

Other methods based on the use of biofilters have also been studied for the recovery of polyphenols from OMWW. Ena et al. (2012) employed adsorbing vegetable and mineral matrices, *Azolla* and granular activated carbon, respectively, with an important capacity for both adsorption and desorption of HT from OMWW. The two matrices showed high antioxidant capacity and antiradical activity.

Overall, biorecovery of OMWW phenolic compounds could not only provide economic advantages, but also could make this wastewater less harmful and easier to treat, thus resulting in OMWW sustainable management (Federici et al., 2009).

#### 6.3. Uses of OMWW in agriculture

##### 6.3.1. Co-composting

The treatment of highly organic OMWW and its use as fertilizer could be regarded among the most suitable and sustainable alternatives in order to manage this waste. This could be ascribed to the fact that through such an application, the nutrients taken up by olive trees cultivation could be returned to croplands (Arvanitoyannis and Kassaveti, 2007).

Composting is defined as a degradation process of solid organic wastes mediated by microorganisms. Such biological decomposition can occur at either aerobic (Makan et al., 2014) or anaerobic (Minale and Worku, 2014) conditions, with the former being more common. During composting, organic matter is transformed through the enzymatic activities of specialized microbial populations, producing a stable and humus-rich complex mixture (Cooperband, 2002; Federici et al., 2011).

During the composting process, factors such as substrates composition, initial C/N ratio, temperature, pH, aeration, porosity, and moisture content must be adjusted in order to provide an optimum environment for the degradation of organic loads, and the humification process. As a liquid, OMWW could not be directly composted but has been employed to produce high-quality composts in combination with saw dust, domestic sewage sludge, manures, and cereal straws among other solid substrates in a process called “co-composting” (Akratos et al., 2017).

Compost is regarded advantageous as a fertilizer agent since it: (i) improves soil water capacity and aggregates stability; (ii) boosts cation exchange; (c) enhances microbial activity, and (d) increases the degradation of pesticides and other synthetic organic substances (Cerdeja et al., 2018). Moreover, the composted material obtained has favorable consequences for both soil and water microbial abundance and diversity (Doan et al., 2014), avoiding drawbacks often observed in direct applications such as phytotoxicity, leaching of nutrients, and inhibition of soil microflora (Felipo, 1996).

Co-composting of OMWW depends on the proper adjustment of pH, temperature, moisture, oxygenation, and nutrients as well as on the adequate development of the microbial populations (Arvanitoyannis and Kassaveti, 2007). Chang et al. (2006) reported that the optimal conditions for an ideal OMWW co-composting process were a starting C/N ratio of 20 to 40, a moisture content of around 50%-60%, an adequate oxygen supply, small particle size, and enough interstitial space through which air could flow.

Controlling microbial populations is essential to understand the composting process of OMWW and to make it successful. However, there are still only a few reports in the literature on this topic. Abid et al. (2007) published a study focused on the analysis of the microbial communities during the composting of an OMWW sludge (obtained by electro-Fenton oxidation of OMWW) in a bench-scale reactor. The dynamics of microbial diversity was followed through a respirometric test and by means of both

cultivation-dependent and cultivation-independent techniques (PCR-single-strand conformation polymorphism; SSCP). During the 7-24 d (the period of high respiration rates), the direct cultivation method showed that thermophilic bacteria and actinomycetes prevailed over eumycetes; however, the PCR-SSCP method showed a higher diversity in the bacterial community than in the eukaryotic ones during the 60 d of the process. Vivas et al. (2009) assessed the changes in the microbial community during composting and vermicomposting (characterized by the addition of earthworms) of an olive-mill waste. They used the real-time PCR assay targeting 16S rRNA genes and the denaturing gradient gel electrophoresis profiling-sequence analysis of PCR-amplified 16S rRNA fragments (PCR-DGGE) in order to determine functional diversity, bacterial number, and bacterial community structure. The results of this study demonstrated that *Proteobacteria* was the most abundant bacterial phylum in both composting and vermicomposting. Additionally, the authors demonstrated that olive mill waste composting and vermicomposting modified the original microbial communities of the olive waste in different ways. Whereas the most representative bacteria in the mature compost (*Actinobacteria* and *Gammaproteobacteria*) were more abundant in olive waste vermicomposting, bacterial phylogenetic groups typical of non-cured compost (*Alphaproteobacteria* and *Bacteroidetes*) were clearly determined in olive waste composting.

Vermicomposting was more effective in activating the microbial metabolism and bacterial diversity of the olive mill toxic waste, probably because *Eisenia foetida* (the earthworms used as a starting inoculum) has a unique indigenous gut-associated microflora which could contribute to and modify the original microbial community (Toyota and Kimura, 2000). In a recent work, El Moussaoui et al. (2017) studied the behavior of microbial biomass in a conventional activated sludge used in the treatment of OMWW. Microbial growth and biomass activity, measured through specific oxygen uptake rate (SOUR), were determined continuously for 70 d. Moreover, the dynamics of aerobic microbial communities of the activated sludge was also assessed using the following as culture media: Plate Count Agar to enumerate aerobic revivable bacteria at 22°C (ARB22) and at 37°C (ARB37), Cetrimide Agar to isolate *Pseudomonas* sp. followed by identification in a special medium King A, Sabouraud Agar Medium for mold and yeast counts, and finally Potato Dextrose Agar supplemented with antibacterial for the cultivation of total fungi. The results revealed the biological treatment of OMWW with an efficient activated sludge system. It was also reported that the microbial biomass showed a good response to the increase in OMWW rate through good growth, a stable physiological state, and an adequate settling capacity of the flocs. Aerobic bacteria ARB22, ARB37, *Pseudomonas* sp., yeast, and fungi concentrations increased significantly from an initial 30% OMWW concentration until the end of the assay.

A stable organic matter content indicates if the compost has a high degree of stability or maturity, is free of phytotoxic elements and plant or animal pathogens, and thus, if it is suitable to be safely applied to soil (Chowdhury et al., 2013). Whereas maturity is associated with phytotoxicity, stability is often related to the microbial metabolism of compost (Iannotti et al., 1993). In turn, stability depends on the relative stability of the composting substrates and on the effect of other physicochemical characteristics of the compost on the growth and development of the plants. This indicates that maturity does not include only a single property, so that it is better evaluated through the measurement of two or more compost parameters. According to the California Compost Quality Council, criteria and parameters for checking compost maturity are based on different physical, chemical, and biological features (CCQC, 2001).

The germination index (GI), a fundamental parameter used to evaluate compost phytotoxicity, is one of the most important indices of maturity. When GI values are close to zero or below 80% in the initial activation stage, they indicate very high phytotoxicity of the composting mixture (Lasaridi et al., 2006). Principally, low GI values could be attributed to the fact that at the starting stage, substrates have high concentrations of water-soluble organic substances, toxic constituents like alcohols, organic fatty acids and phenolic compounds, elevated C/N ratios due to the presence of ammonia and other toxic nitrogen-based products, as well as high heavy metals and mineral salt contents (Said-Pullicino and Gigliotti, 2007). After the maturation phase, the GI increases to values ranging from a minimum of 66% (Gigliotti et al., 2012) to a maximum of 201% (Zorpas and Costa, 2010), thus surpassing the threshold limit of 80% that determines the phytotoxicity of the compost (Zucconi et al., 1985). A relatively high GI recorded at the end of the composting indicates that

OMWW could be converted into a high-value soil amendment. Makni et al. (2010) composted OMWW and obtained final GI values above 80%, so that the final compost could be characterized as mature or very mature. The effectiveness of OMWW composting as a recycling technology in agriculture depends mostly on the quality of the compost; consequently, the characterization of the process plus the evaluation of the quality of the mature composted material are crucial (Cayuela et al., 2008a and b).

### 6.3.2. Fungal biomass and edible mushrooms

OMWW has long been used in the preparation of production media for the development of certain microorganisms with the aim to obtain potentially edible microbial biomass. Edible fungi, especially *Pleurotus*, *Lentinula*, and *Agaricus* species, were able to grow using olive mill wastes and wastewater as nutrient sources, by applying different strategies (Altieri et al., 2009; Koutrotsios et al., 2016). Kalmis et al. (2008) cultured the edible mushroom *Pleurotus ostreatus* on wheat straw substrate mixture with tap water and increasing concentrations of OMWW in order to investigate the feasibility of using OMWW as an alternative wetting agent and as an eco-friendly solution for the commercial production of mushroom. With a 25% OMWW (v/v) concentration, the experimental results showed positive effects on mushroom growth. Increasing volumes of OMWW showed negative effects, including lower bio-efficiency and deformation of fruit body shape. Lakhtar et al. (2010) screened sixteen strains of *L. edodes* but pre-selected only four of them (Le118, Le119, Le121, Le122) due to the higher biomass yields obtained when grown in the presence of 20% (v/v) OMWW. Laconi et al. (2007) implemented a combined chemical-biological process (alkaline oxidative treatment to decrease polyphenols content of OMWW linked to a fungal fermentation) in order to obtain intense degradation of pollutants in OMWW coupled to the production of high-rich microbial biomass (mixture of edible fungi genus *Pleurotus* together with the yeasts *S. cerevisiae*, *Kluyveromyces lactis* and the species of filamentous fungi *Oidodendron* spp. and *Penicillium* spp.), which could be used as an animal feed additive. Up to 150-160 g of wet biomass with a protein content of about 13 g percent and 6 g percent of raw fiber were obtained per liter of treated OMWW.

In a recent study, Koutrotsios et al. (2016) evaluated the suitability of water diluted-OMWW (12.5%, 25%, and 50% v/v) as a substrate for the production of *H. erinaceus* biomass. The *H. erinaceus* most abundant mycelium was obtained in 50% OMWW, achieving  $154.80 \pm 8.45$  mg/100 mL.

Additionally, *P. ostreatus* presented satisfactory growth and reduced the phenolic content of sterilized OMWW in bioreactor cultures. However, high OMWW dilutions and/or additional treatment were still needed before the treated OMWW could be discharged into the environment, e.g., as water for irrigation (Aggelis et al., 2003). The biological evaluation of the treated OMWW as water for irrigation of plants growing in pots did not significantly affect the uptake of various nutrients, but plant yields were decreased, probably due to the high OMWW salinity (Aggelis et al., 2003).

### 6.4. Another use of OMWW: fiber restoration for animal feed and food production

The increasing fiber consumption trend has led to the development of a large potential market for fiber-enriched food products, ingredients, and gelling materials. Olive mill by-products contain olive cell wall polysaccharides debris and have been proposed as a source of polymers such as pectins, hemicelluloses, cellulose, lignin, as well as other products like gelling agents and fat replacements (Galanakis, 2010). Dietary fiber is defined as the traces of edible plant cells and consists of all the above substances plus other associated ones that are resistant to digestion by human enzymes (García et al., 2007). Although all these polysaccharides are present in olive mill wastes, the co-presence of phenolics and other organic matters hinders their extraction and purification and demands costly equipment, processes, and chemicals. OMWW and olive pomace are rich in pectic materials but they could not be transformed into an exploitable source of gelling agents because of their rich phenols contents (Cardoso et al., 2002). In this sense, efforts have been made to optimize the functional properties of these pectic polysaccharides. In a recent research, selective lignin-degrading fungi and solid-state fermentation were used in an effort

to improve the nutritional properties of an olive mill residue mixed with other feedstuffs (wheat bran, wheat middlings, barley grains, crimson clover, wheat flour shorts, and field beans) by using the macrofungi *P. ostreatus* and *Pleurotus pulmonarius*. Between 50% and 90% of the phenolic content was removed from the waste and its crude protein content was increased by 7–29% after 6 weeks (Brozzoli et al., 2010). Nevertheless, fiber production for either animal feed or food with a commercial interest using olive by-products as cheap and abundant sources seems feasible and competitive within the near future if the cost of treatments to eliminate their phenolic contents could be decreased (Federici et al., 2009; Dermeche, 2013).

## 7. Concluding remarks and future prospects

Olive oil represents one of the most important agricultural commodities. However, olive oil manufacturing leads to severe environmental degradation when OMWW is produced, discarded, and its management is not adequate. No individual treatment technology has proven suitable enough to be adopted by an olive oil-producing factory. Currently, olive processing-derivate wastes are either impounded in storage lagoons or discharged into receiving media, but an advisable treatment requires a multifactorial approach, combining a biological step that might integrate more than one type of bio-treatment with innovative process engineering to handle residues and derived compounds.

In accordance with the biorefinery concept, all sustainability features including economic, environmental, and social elements with an emphasis on the production of highly value-added compounds should be taken into account. In better words, this concept requires the appraisal of the whole biomass in terms of carbon and energy, based on a zero-waste notion.

In light of the above, the future perspectives of the olive oil industry should be based on a biorefinery framework through which the possibility of using various waste streams including OMWW as renewable raw materials to generate high value-added products be explored. To achieve that, different biotechnological production processes including combined and/or sequential treatment schemes should be developed and implemented. The various high value products generated through such platforms ranging from bulk fertilizers and other soil amendment products to more specialized ones such as antioxidants, catalysts, biofuels and biochemicals could significantly enhance the economic viability of the whole industry while simultaneously reduce its footprints. On the other hand, this would constitute a viable and safe solution for the environmental problems associated these waste streams.

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